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B. T. GALLOWAY, Chief of Bureau.

OBSERVATIONS

ON

THE MOSAIC DISEASE OF TOBACCO.

BY

ALBERT F. WOODS, Pathologist and Physiologist,

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., January 25, 1902.

Sir: I have the honor to transmit herewith a paper on Observations on the Mosaic Disease of Tobacco, and respectfully recommend that it be published as Bulletin No. 18 of the Bureau series. The paper was prepared by Mr. Albert F. Woods, Pathologist and Physiologist, Vegetable Pathological and Physiological Investigations.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

The work described in this paper was started during the winter of 1898–99, continuing as other work permitted to the latter part of December, 1900, when it was brought together in its present form and presented December 27, 1900, at the Baltimore meeting of the Society for Plant Morphology and Physiology. Since that time the writer has been unable on account of other work to continue the investigation. It is believed, however, that the true nature of the mosaic disease of tobacco and of similar diseases of other plants has been found and that it will be possible in large measure to guard against them. Further investigation of some of the points left unsettled is at present in progress. The writer hopes that these may soon be settled with the help of Dr. R. E. B. McKenney, who has been assigned to the work.

ALBERT F. WOODS,
Pathologist and Physiologist.

OFFICE OF THE PATHOLOGIST AND PHYSIOLOGIST,
January 23, 1902.

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This very singular disease of the tobacco leaf consists, in general appearance, in a more or less sharply defined differentiation into light and dark green areas, making the leaves have a more or less mosaic appearance. In the less pronounced cases the variation in the color is slight, but sufficiently marked to be at once apparent. In such cases there is very little distortion of the leaves. The light-green areas are, as a rule, between the larger vascular bundles. (Pls. I and VI.) The darker green portions form more or less of a border along the larger bundles. Occasionally, however, the dark and light green patches are not distributed with special reference to the vascular bundles, but occur indiscriminately. Where the contrast is more marked the light-colored, or sometimes even yellowish, areas grow slowly, while the dark-green areas grow more rapidly, and thus the leaf becomes badly distorted. (Pls. III and IV.) In some cases the whole plant becomes so deformed as to be almost unrecognizable. Even where the leaves are only slightly diseased and not deformed they are less elastic than the healthy leaves, and are not so suitable for wrappers for cigars, and have a poorer burn and aroma. The diseased plants do not grow as vigorously as healthy ones, but they usually produce flowers and fruit, and the seed, even from badly diseased plants, may produce healthy plants. There is no conclusive evidence that the plants from seeds of diseased plants are more subject to the disease than are those from the seeds of healthy plants.

The disease occurs more or less throughout the tobacco areas of this country and is widespread in Europe wherever tobacco is grown. As usual there are numerous theories in regard to its cause, many of which it will not be necessary to discuss here. Adolph Mayer[a] was the first to make a careful study of the trouble. He demonstrated that it could not be caused by an insufficient supply of mineral nutrients. He found as much nitrogen, potassium salts, phosphates, calcium, and magnesium present in the soils and plants where the disease occurred

[a] Landw. Versuchsstation, 1886, Vol. XXXII.

as in the soils where the disease did not occur. He also found that the trouble was distributed over a field apparently without regard to soil conditions.

Since tobacco requires much lime, liming of the soil was tried, but the disease was not prevented thereby. Mayer further kept hotbeds in some cases rather moist, in others dry, and then again richly or poorly manured with nitrogen; but in none of these cases could he determine that the conditions in question caused the disease. He also found that variations in the temperature of the hotbeds apparently had no effect; neither did crowding, which produced partial etiolation, appear to have any effect on the disease. Seeds from flowers in which self-fertilization was prevented he found to be just as susceptible to the disease as seeds produced without such precautions, but on soil on which the disease had once appeared it was again produced. According to his observations, also, the trouble was not often found on soil used for the first time for tobacco. He further proved that the juice of the diseased leaves injected into the growing parts of healthy tobacco produced the disease in the inoculated plants, while control plants injected with the juice of healthy plants did not develop the disease. He was also not able to produce it by injecting diseased juice into other solanaceous plants. Where the diseased juice was injected into tobacco the same trouble developed in from ten to eleven days. Heating to 60° C. did not destroy the infectious substance; at 65° C. to 75° C. it was attenuated, and at 80° C. it was killed.

After Mayer had shown the absence of animal and fungous parasites he supposed bacteria to be the cause of the disease, but all his efforts with bacteria cultivated from the surface of diseased leaves, and also with different mixtures of bacteria, failed to produce it. Nevertheless he thought that there must be certain pathogenic bacteria in those soils in which the disease appeared, and therefore proposed to change the soil in hotbeds and to devote the fields where tobacco had been cultivated and the disease had appeared to other crops. He also recommended the use of mineral rather than organic manures.

These general results were confirmed by several subsequent investigators. Not, however, until Beijerinck[a] took hold of the question was much of importance added to our knowledge of the malady. He proved the absence of bacteria in the development of the disease. He showed that the juice of diseased plants filtered through Chamberland filters, while remaining perfectly clear and free from bacteria, still retained the power of infection. A small drop of it injected hypodermically into a growing bud was sufficient to give the plant the disease. He found that only dividing (meristematic) cells can become diseased. Diseased tissue kept its infectious qualities even after dry-

[a] Verhandelingen der Koninklijke Akademie van Wetenschappen te Amsterdam, 1898.

ing, and retained its injurious properties in the soil during the winter. Weak solutions of formalin did not kill the virus, but heating to the boiling point did. Fresh, unfiltered juice was more effective than an equal amount of filtered juice. He found that soil around diseased plants may infect the roots of healthy plants, but he did not determine whether direct transference is possible through healthy root surfaces, or whether insects, by injuring the roots, favored infection. He defines the milder form of the disease as a suffering of the chlorophyll bodies. Later on a general disease of the entire plasmatic contents of the cells sets in.

In field conditions as a final stage the swollen green areas became marked with small dead spots, but these did not appear in plants grown under glass. Under certain conditions he observed that plants apparently recovered from the disease; that is, the new growth appeared to recover. He found that the infective material, whatever it might be, could be transported through considerable distances in the plant, but could cause the disease only in dividing cells. He assumed the virus to be a noncorpuscular, fluid-like material, which had the power of growth when in contact, in a sort of symbiotic way, with growing cells—"a living fluid contagium."

Shortly after Beijerinck's paper, Sturgis published a critical review of all the work done on the disease up to that time, with numerous valuable observations made in Connecticut, where the trouble is known as "calico," or "mottled top." He presents the following summary of his observations and conclusions:[a]

1. The peculiar appearances known as "calico" and "mottled top" of tobacco are probably symptoms of one and the same disease. The former may occur very early in the life of the plant, even in the seed bed, and usually attacks first the older leaves. The latter occurs later, is less pronounced, and affects only the topmost leaves.

2. The disease occurs abundantly in some localities, notably on the close, clayey soils on the east side of the Connecticut River; sparingly in other localities, where the soil is open and porous.

3. The disease is not contagious. As to its infectiousness, no direct statement can as yet be made.

4. It is not caused by predaceous insects, nematodes, or parasitic fungi.

5. Bacteria have not been seen associated with the disease, but no critical method for their isolation or culture has been applied, and therefore the question of their influence can not at present be answered. The facts observed, however, are not favorable to the theory of bacterial infection.

6. The disease is not inherent in the seed. Seed from badly calicoed plants may produce perfectly sound plants, and vice versa.

7. It seems probable that the disease is purely a physiological one, caused primarily by sudden changes or atmospheric conditions which disturb the normal balance between evaporation of water from the leaves and its absorption by the roots, and secondarily by soil conditions which prevent the speedy restoration of that balance. This supposition is supported by numerous facts.

[a] Conn. Expt. Sta. Report for 1898, pp. 252, 253.

Koning, as a result of his investigations of the trouble, very largely confirmed the work of Beijerinck and others.[a] My own study of the disease, briefly described in Centralblatt für Bacteriologie, Parasitenkunde, u. Infektionskrankheiten, Abt. 2, Band V, 1899, also agreed in the main with what I have already outlined as observed by other investigators. I pointed out in addition that the mosaic disease of tobacco was always accompanied by an excessive quantity or an excessive activity in the diseased cells of an enzym belonging to the oxidases, agreeing in this and some other important particulars with the changes known as variegation and albinism in various other plants, and often developing into this malady in tobacco. In all of these cases careful comparative investigation showed that the power of oxidation in the cells is inversely proportional to the amount of chlorophyll present, judging by color. Somewhat later I pointed out[b] an important structural difference between the cells of the green areas and those of the light. In the latter, in the less pronounced cases of the disease, there is a shortening and broadening of the palisade parenchyma cells, and in the more pronounced cases there is an entire suppression of these cells, so that on simply looking across the surface of the leaf depressions are seen where the light areas occur and apparent blister-like development in the green areas (Pl. I). As a rule the modified cells pass abruptly into the normal cells of the green areas. The palisade cells of healthy leaves and of the healthy areas of diseased leaves are from three to four times as long as broad. Some plants are dark green, and these have the longest and narrowest palisade cells, closely packed with dark-green chloroplasts, which seldom contain large starch grains. Other plants are much lighter in color. These have relatively broader palisade cells, approaching more in size and shape the spongy parenchyma cells of the same leaf. In both the light and the dark leaves the spongy parenchyma cells are about isodiametric, the diameter being about twice the length of the contiguous palisade cells in the mature leaf. In both the dark and the light leaves the number of chloroplasts is somewhat greater in the palisade cells than in the spongy cells, but their shape, size, and intensity of color are apparently the same in the same leaf. The wider palisade cells of the light type of leaves permit the light to pass through them more readily, the spaces between the chloroplasts being larger and the intercellular spaces fewer.

In comparing light and dark leaves there was also found a slight difference in the intensity of the color of the chloroplasts. Less green can be extracted from a given area of light leaf than from the same area of dark leaf, and this difference is also apparent in microscopic examination of the chloroplasts. The light-colored leaves show a

[a] Zeitschrift für Pflanzenkrankheiten, Band IX, Heft 2.

[b] Science, n. s. 11, No. 262, pp. 17–19.

greater tendency to store starch than do the darker ones, and they are slightly richer in oxidizing enzyms. In these particulars, therefore, they suggest a variation toward a condition found in moderately diseased cells. I have not observed, however, any greater tendency in these light-colored plants to develop the true mosaic appearance either naturally or under artificial manipulation. That the disease is not primarily of the chloroplasts, as Beijerinck thought, is evident from the fact that in the less pronounced cases the chloroplasts, though fewer in number, are not decreased in size or activity. However, the starch which they make is, as I pointed out in the paper cited, not readily converted into sugar, and hence is translocated with great difficulty. In some pronounced cases the chloroplasts are light-colored, or are wholly without color. This condition, however, appears to be a result rather than the primary cause of the diseased condition of the cells.

The conditions which cause the disease are certainly only effective when acting on meristematic or dividing cells. It can not be induced artificially, and never appears naturally, in cells which have stopped dividing. A diseased spot, therefore, never increases in size in a leaf except as the diseased cells themselves enlarge. The mosaic nature of the trouble and the fact that under some conditions the plants may grow out of the disease suggests that it must arise in such cases in the meristematic tissues behind the growing point, or, where the plant is not mosaic, but evenly diseased, the pathological condition must be present throughout the cells of the growing point.

TRANSLOCATION OF STARCH.

Examination[a] of the diseased spots early in the morning shows only a small decrease in the starch content of the cells from that present in them the previous afternoon, while the green, healthy tissues contain no starch, or only traces of it. It was thought that possibly the increase of oxidizing enzyms might either inhibit the production of diastase of the cells or inhibit the action of diastase upon starch. In order to settle this point strong solutions of the oxidizing enzyms of tobacco were prepared from diseased plants growing in the greenhouse in December, and after heating some of the solution to the boiling point, thus killing these enzyms, comparisons of the heated and unheated juice were made by adding 10 milligrams of taka diastase in solution to each of the tubes of juice to be tested. Equal quantities of freshly prepared potato-starch paste were then added to each tube and the tubes kept at 45° C. It was found that in a solution without

[a] The most favorable method of examining the leaf for starch is to kill it in hot water, decolorize with alcohol, and immerse in iodine-potassium iodide solution. Very good results are obtained by immersing one minute in boiling water, transferring to cold water, then to the iodine solution, and examining by transmitted light.

oxidizing enzyms the starch was completely converted into sugar in thirty minutes, while the solutions in which these enzyms were active carried the change of the starch only to the erythro-dextrin stage in the same time. The action of the diastase of malt added in solution in the same quantity was somewhat less rapid than that of taka diastase, but the relative effects were the same, i. e., the presence of the oxidizing enzyms in solution had a marked inhibitory action on the activity of the diastase.

In these tests the amount of diastase compared with oxidase was much greater than occurs in the diseased cells, so it is likely that the inhibitory action of the oxidase in the cells is much greater than that shown in the tests outside the cells. It would seem a warrantable conclusion, therefore, that in plants grown under glass in winter the tardiness in the translocation of starch in the diseased areas is to be explained by the abnormal activity of the oxidizing enzyms of these cells, and that the mode of this action is by retarding or weakening the activity of the diastase. This action of the oxidizing enzyms upon the diastase therefore retards the production of sugar and, consequently, of proteids and reserve nitrogen in general. It is probably on account of this that the cells of the diseased areas are relatively very poor in reserve nitrogen.[a] The slower growth of the diseased areas is most probably the result of this decrease in the availability of reserve organic materials—sugar and proteids. Whether this holds for the summer-growing periods is still to be determined. The inhibiting action is probably much less marked during warm weather.

ARTIFICIAL PRODUCTION OF THE DISEASE.

I have already pointed out that the mosaic disease may be easily produced by removing the top of a rapidly growing plant in any stage of growth. A few buds at the base of the stock must of course be left, and it is best also to leave a few leaves. The shoots that develop after cutting are, in practically every case, badly diseased. During the past year I have produced the disease in this way in plants only 4 inches high in the seed bed, and also by cutting back fruiting plants 3 to 4 feet high. Over 200 plants of various ages were cut back, and the new growth came diseased in every case. I have also experimented in the same way with tomatoes, potatoes, petunias, English violets, pokeweed (Phytolacca), and other plants, and find that the same disease can be produced in them by removing their tops and forcing sprouts to grow from the stems. Sometimes one cutting back is sufficient to produce a well-marked case of the disease, but usually in the tomato and potato two removals of the top are necessary. Plates II,

[a] Loew. Physiological Studies of Connecticut Leaf Tobacco. Report 65, U. S. Dept. of Agr., pp. 11–25.

V, and VI show the disease produced in tomato and Phytolacca in this way. In these plants, as in tobacco, the diseased cells are greatly retarded in growth. They are rich in oxidizing enzyms, poor in proteids (soluble nitrogen), and have a tendency to store their starch rather than to convert it into sugar and translocate it. The most important thing that at once suggests itself in connection with the cutting back is the removal by this process of the large amount of reserve material that furnishes the food for roots and new growth.

When the top is cut off the small amount of the available reserve food left in the remaining parts would be expected to migrate in the direction of the demand of the roots. A new shoot which starts has to furnish not only its own organic food, but is drawn upon by the roots. It, of course, has an abundant supply of water and soil nutrients, but it is lacking in sufficient organic food—sugar and proteids—to meet the demands of the rapidly developing cells. The young dividing cells can not make direct use of the ordinary soil nitrates. Nitrate of potash, for example, may accumulate in a plant under various conditions, such as insufficient light, lack of the proper development of the chloroplasts, as in variegated plants, etc. Overfeeding with nitrates often causes a yellowing of the chloroplasts, probably by the accumulation of unused nitrates in the cells. A similar condition of food distribution may also develop in the formation of sprouts where they occur on a rapidly growing plant. In such cases they are as a rule badly diseased. When mosaic sprouts once develop on a plant all the new growth afterwards is likely to be mosaic, though of course the leaves already formed are not affected and the plant may not be commercially injured, as the sprouts and top will be cut away. Sometimes a perfectly healthy plant produces a few mosaic leaves at the top just as the flower stems begin to develop. This suggests the possibility that the developing flower stems get most of the organic food during the first stages at least of the development of these leaves. Removal of the flower buds, even if done at the first appearance of the disease, does not cure the trouble, though the leaves will make a much more vigorous growth than they otherwise would. In such cases, after the removal of the flowering buds, there is a tendency to produce flowering suckers. These always come diseased. It is very evident, in such cases as I have just described, that the trouble *can not be due to parasites and must be attributed to a disturbance of the normal physiological activity of the cells in question.* That this disturbance is primarily one of nutrition is indicated by facts already pointed out and others to be presented in the following pages.

Prof. U. Suzuki, Imperial University of Japan,[a] has made a very careful study of the so-called mulberry dwarf troubles in that

[a] Bulletin of the College of Agriculture, Imperial University, Tokyo, IV., No. 3, July, 1900.

country. As it is evident that the trouble is related to the one under discussion here,[a] I quote in the following paragraph from his report on the subject. It is the custom in Japan to cut back the mulberry trees each year to stimulate a new growth of branches and tender leaves for the silkworm. There are three principal methods of cutting: Low cutting close to the ground; medium cutting, leaving stumps about a foot high; and high cutting, leaving stems about 6 feet high. The cutting is done about the third year of the life of the plant, and usually in June, at the time of the fullest development of the leaves.

> By August or September the new shoots reach a height of 5 or 6 feet, and these are again cut down in May of the next year. This treatment secures a large crop and lessens the injury from insects and fungi. * * * The first sign of the disease usually appears on new shoots springing from stumps. When these have reached a height of 1 foot or so the upper leaves either begin to shrivel or manifest other signs of debility; and, as the shoots continue to lengthen, all the new leaves developed from them betray the same character. The diseased leaves may turn yellowish or remain a dirty green. * * * In acute cases the leaves may all shrivel up in one year, but usually only a few leaves near the top of the shoot betray their debility, whence the disease spreads with each successive cutting, until in the course of a few years the entire plant is attacked or even dies off. The branches of the attacked plants usually remain slender, and the twigs and leaves are very numerous. * * * Sometimes also the branches lose their strength and become procumbent. The unfailing signs are the imperfect development and shriveling of the leaves and the slenderness and dwarfed condition of the branches. Moreover, when a plant is once attacked recovery is possible only after two or three years of complete protection from cutting.

After an extended examination it was found that the trouble was largely confined to plants that had been subjected to low cutting, as described. It occasionally, however, developed on young plants which had never been cut, and sometimes on older plants which had never been subjected to cutting. These cases were very rare, but the cause of the disease in these was found to be due to a lack of sufficient reserve food to meet the demands of the growth. How this came about in the absence of cutting was not definitely determined, though it is clear, as the result of experiments which I will speak of later, that it might be caused by defoliation following insect or fungous attacks.

By an extensive series of analyses it was found that during the period of growth of the mulberry the reserve materials, principally the nonalbuminoid nitrogen (amido compounds soluble in water), in the bark of roots and stems are all transported to the growing parts and reach the minimum, and therefore if the plants are cut down in this period the new shoots are forced to depend on an exceedingly

[a] Dr. Loew, in a letter dated December 12, writes as follows: "The observation that you made that oxidase can kill diastase has been also made by my assistant (Suzuki) in the case of mulberry leaves. Diseased leaves have more oxidase than healthy ones and no longer transport starch." Cf. Bul. Col. Agr., Tokyo, Vol. IV, No. 4.

small quantity of reserve food; hence the leaves are imperfectly developed and the reserve materials are exhausted before the plants can absorb and assimilate adequate nourishment from the soil and the atmosphere. Professor Suzuki therefore concludes that the principal cause of the disease is to be sought in the practice of subjecting the mulberry to repeated low cutting, thus removing the reserve food required in growth. He also found that frequent picking of the leaves exhausted the reserve materials in the stems and caused new leaves to become diseased. He determined that some varieties of mulberry store large quantities of reserve materials and others very little. The former are quite resistant to injuries from cutting back and by the removal of leaves, while the latter are very easily injured in these ways. Winter cutting of dormant wood does not produce the disease, because sufficient reserve food is left.

Conditions, such as the abundance of soluble manures, favoring too rapid growth, are very favorable to the disease. The application of nitrogenous or other quick-acting manures does not cure the disease, but intensifies it. This is also the case in the mosaic disease of tobacco.[a] All of the evidence in this case, therefore, points to a condition in the cells of the diseased mulberry very much like that in the mosaic disease of tobacco, tomato, potato, Phytolacca, and other plants. In the case of the mulberry it appears to be demonstrated beyond a doubt that the trouble is primarily due to growth, with an insufficient supply of elaborated nitrogen as available food. Under these conditions there should be found in the diseased mulberry an accumulation of oxidizing enzyms, which in part by their action on the diastase of the cells inhibit the translocation of starch or its transformation into sugar and proteids, thus serving to intensify and fix the disease in the plant.

It thus appears that *whatever causes the reduction of available nitrogenous reserve food, especially soluble nitrogen, below the requirements of dividing cells may cause this disease in the tissues in question.* All of the cases of the disease in tobacco and other plants produced by cutting back severely can be thus explained. We have also a pathological condition of the same nature produced by sucking insects and mites in the young growth of carnations and other plants. Large quantities of reserve proteids and sugars are thus removed, the oxidizing enzyms increase, and the growing cells lose their chlorophyll and finally die. I have described these changes under the name of stigmonose of carnations.[b] The occasional appearance of the disease, as before mentioned, in the young leaves produced at the time of the development of flowers can be explained in this way, as can also the development of the disease in suckers forming on rapidly growing plants.

[a] Loew, l. c., p. 26.

[b] Bul. No. 19, U. S. Dept. Agr., Div. Veg. Phys. and Path.

The disease is often produced by repotting the young, rapidly growing plants or by transplanting them. At first it was thought that this was due in some way to possible infection from diseased plants, but the work of the past year under conditions free from any possible infection has demonstrated that the trouble can often be induced simply by repotting or transplanting. A rapid root development is induced by this process and it is likely that the reserve nitrogenous food for the terminal growing bud of the stem may be sufficiently reduced to start the trouble. The continuation and intensification of the disease after it is once started is probably to be explained by the action of the oxidizing enzyms, as before pointed out.

INFECTIOUS NATURE OF THE DISEASE.

The apparently infectious nature of the malady is, however, difficult to explain in accord with the facts presented unless the oxidizing enzyms artificially introduced into the plant have the power of inaugurating changes like these described. In my former paper in the Centralblatt f. Bakteriologie, already cited, I pointed out the conflicting results of my first inoculation experiments. I did not at that time obtain infections with the juice of diseased plants filtered through porcelain; and the results obtained by inserting pieces of diseased tissue, healthy tissue, and simple wounding were also apparently conflicting. In one experiment, for example, the disease was produced by inserting a piece of healthy leaf into the stem of a healthy tobacco plant below the terminal bud; in another case the disease developed by simply splitting the stem, without inserting any tissue, while in two cases where diseased tissue was inserted the plants remained healthy. In another experiment the disease was produced by injecting a sterile water solution of peroxidase (obtained by alcoholic precipitation from the juice of a healthy plant) into two young shoots of another healthy plant. These shoots became very severely diseased and distorted, while other shoots on the same plant, as well as those on control plants treated in the same way with distilled water, remained perfectly healthy.

During the past year, however, the results have been less conflicting, and I have come to the conclusion that the failure to produce the disease in some of my former experiments was due to the fact that the diseased tissue was not inserted close enough to the base of the terminal bud. In a recent experiment 100 seedling plants growing in the seed bed were selected. They were about 6 inches high and had from 6 to 8 leaves well developed. All were perfectly healthy. Fifty of the plants were from seed which had been produced by artificial self-pollination, and 50 from seed by artificial cross-pollination. The inbred plants were of light color and the cross-bred of darker green color. So far as determined, inbreeding had nothing to do with the

production of the light color. Ten plants (5 each of the light and dark sorts) were selected and their stems split just at the base of the terminal bud; 10 (5 light and 5 dark) were split in the same way and a piece of minced terminal bud from a healthy plant inserted in each, and 10 (5 light and 5 dark) were split and a piece of minced diseased bud inserted in each. In eight days all of the 10 plants infected with diseased material developed violent cases of mosaic disease; not any of the other plants showed signs of the disease at that time. One week later, however, 8 of the 10 plants in which the healthy tissue was inserted were affected, though not so severely as where diseased tissue was used. Not any of the plants which simply had their stems split with the sterile scalpel developed the disease. All were grown to maturity without transplanting. None of the controls developed the disease, and none of the diseased plants recovered, though all produced a few flowers and seed. The plants were fed with nourishing solutions, but did not grow large on account of being too close together. It should be especially noted that, though they were growing so close together that their roots were interwoven, the disease did not spread to the healthy plants. This agrees with field observations, where the plants are often found in pairs, one healthy and the other diseased.[a]

The next experiment was with similar plants from seed out of the same pods as in the experiment above described. There were 30 controls and 30 plants operated upon. They were growing in 3-inch pots and had from 6 to 8 leaves at the time of the experiment. All were healthy. The value of the results was interfered with by the use of lime on the bench under the pots to drive away slugs. Some of the plants were so badly injured in this way that they died. One important point was thus determined, however, namely, that the lime in this case had no effect, one way or the other, upon the development of the disease as the result of inoculation. Injection of unfiltered diseased juice into the terminal bud produced the disease in eight days in 4 out of 5 of the plants treated. The other plant was killed by lime. The disease was also produced in 3 out of 5 cases by pouring the diseased extract on the roots at the base of the stem. The other 2 plants of this series were killed by lime. Boiled diseased juice also produced the disease in 2 out of 5 cases when inj cted into the growing plant. The other 3 plants were killed by lime. Boiled diseased juice also produced the disease in 2 cases in eight days when poured on the roots. The other 3 plants were killed by lime.

ZYMOGEN FOR OXIDASE AND PEROXIDASE.

The boiled diseased juice just mentioned stood eighteen hours at 60° C. before use. This temperature for the time mentioned does not injure the oxidizing enzyms, but prevents bacterial development. It

[a]Loew, l. c., p. 26.

was tested immediately after boiling and found to be free from the enzyms in question. The next day after the injection experiments the remaining portions of the boiled juice were tested and to my surprise gave a strong reaction for both oxidase and peroxidase: in fact it was scarcely weaker than the reaction of the unboiled juice. This at once suggested the possibility of a zymogen for these enzyms. Upon further study, this supposition was found to be correct. In the leaves of tobacco themselves and in the unfiltered extracted juice both enzyms are regenerated in two hours after boiling. A second boiling after four hours is not followed by a regeneration of the enzyms. It is evident, therefore, that the zymogen exists in the cell in sufficient quantity to regenerate practically the same amount of active enzym as is already in the cell. The transformation of the zymogen into the active enzym takes place whenever the active enzym in question is removed or destroyed. The relation of the active and the reserve enzym is evidently a balance not controlled by the protoplasm, as the regeneration occurs in cells which have been killed, but of course no new supply of zymogen is manufactured in such cases, even in expressed juice or in the cells of the dead leaf. The zymogen becomes active only in proportion to the decrease of active enzyms present in the cells at the time they were killed. All of the experiments, therefore, with boiled juice, where it was intended to destroy the enzyms, must be looked upon as simply experiments with the attenuated enzyms, or more correctly, perhaps, an enzym solution of half the strength of unboiled juice.

In the remaining 10 plants in the experiment above referred to, those injected with healthy juice became diseased in 2 cases, 2 remained healthy, and 1 was killed by lime.

Where the juice of the healthy plants was poured on the roots, 2 cases of the disease were produced, 2 remained healthy, and 1 was killed by lime. Five of the control plants were injected with distilled water, but none of them developed the disease. Of the remaining 25 controls, 4 were affected with the disease without any apparent cause.

On the whole, this experiment indicates that there is something in the juice of normal tobacco plants which can, under certain conditions, cause a development of this disease. There is a strong indication in the experiment just described that this substance may be an oxidizing enzym.

In another experiment 24 plants were selected. They were 6 to 8 inches high, had 4 pairs of leaves, and were growing in 3-inch pots, all being perfectly healthy. Of these, 4 were injected in the terminal bud with distilled water, 4 with double-boiled diseased juice free from active or reserve enzyms, 4 with a water solution of oxidase and peroxidase obtained by alcoholic precipitation from the juice of diseased plants, and 4 with unboiled diseased juice: there were 8 controls.

In nine days all the plants injected with solution of the precipitated enzyms, and those with unboiled juice, showed the disease decidedly in their young leaves. Examination the day previous showed in some cases an indication of disease, but it was not sufficient to make the diagnosis sure. The suddenness of the appearance of disease on the ninth day in the two sets of plants is quite remarkable. All of the other plants were at this time apparently perfectly healthy; five days later, however, 2 of the plants injected with distilled water, 1 of those injected with double boiled juice, and 3 of the 8 controls became diseased. All of the plants were carefully repotted three days after the experiment was started. This was made necessary because the roots were coming through the bottom of the pot. This last attack of the disease can probably be attributed to the repotting, which, I have before pointed out, is often alone sufficient to cause the disease to develop. These cases among the control plants were not so severe as those produced by inoculation, but were not otherwise different.

No more cases developed in these plants. They were allowed to go on and fruit without further repotting. The evidence again points strongly toward the enzym as an active agent in bringing on the disease as the result of inoculation. As I pointed out in a former paper, the so-called peroxidizing enzyms remain for a long time active in soil containing the decaying roots of tobacco and other plants. The enzym is freed through the process of decay. Beijerinck and others have shown that soil in which diseased plants have been growing is very favorable to the development of the disease in later crops. An experiment was planned to test infection in this manner. Four 7-inch pots, 2 with mature healthy and 2 with mature diseased plants, were selected. The tops were cut off in each case and all the small roots broken up and mixed with the soil from which they were taken. Some well-rotted cow manure was added to each pot. Healthy young plants were then taken from the seed bed with sufficient earth so as not to disturb the roots and were set, 2 in each pot. The plants had only 2 leaves besides the cotyledons, so it was possible to move them without disturbance. This first series, with 1 healthy control pot, was kept in the laboratory, and the amount of light the plants received was very small compared with what they would have received in the greenhouse. Besides the above, 2 similar pots of diseased plants and 2 of healthy were selected and the tops and taproot were removed in each case, but the soil was not otherwise disturbed, and no manure was added. As controls, 2 pots with fresh soil mixed with manure were prepared, and young plants were set in the same way. This last series was kept in the greenhouse, except 1 of the controls, which was added to the series in the laboratory. Besides the young plants transplanted, 5 fresh tobacco seeds were planted in each pot of both series, all of these seeds coming from 1 pod of a healthy

plant. The transplanted plants grew a little faster than those remaining in the seed bed, probably because they were less crowded. The plants in the laboratory grew very slowly compared with those in the greenhouse. After they had grown for a week a sharp scalpel was thrust into the soil near the stem, so as to cut some of the roots of 1 plant in each pot of both series. In the 4 pots in which the diseased roots were present the plants with roots cut developed the disease in eight days after cutting. Those with uncut roots became diseased one after another, until in three weeks all of them were affected. Four weeks from the time of planting the plants with roots cut, growing in soil containing healthy roots, became diseased, and 2 of the plants which did not have their roots cut became diseased a week later. The 2 remaining with roots uncut also finally became diseased. The plants growing in control soil not containing decayed roots remained healthy. The seed sown in the pots nearly all germinated and grew well, especially in the greenhouse. In the laboratory series containing minced roots of diseased plants, one seedling developed the disease in the first leaf after the cotyledon. None of the other seedlings showed any signs of the trouble at this time. This plant, with a healthy one growing near it, was dug up, and 1 of the principal feeding roots of the diseased plant was found growing lengthwise through a piece of decaying diseased root. The roots of the healthy seedling were not in connection with any piece of diseased root in the soil. A test of the soil water, however, indicated the presence of peroxidase, though not in large quantities. The young root growing through the diseased decaying root must have absorbed much more peroxidase than would have been possible if it had not been connected with the decaying root. The diseased piece of root was removed, and was found on examination to be rich in peroxidase. Both plants were again planted, but the diseased plant did not recover.

In the greenhouse all of the 5 seedlings in 1 of the pots in which a diseased plant had been growing (the soil remaining full of decaying roots) became diseased in the first pair of leaves after the cotyledons. In the other pot containing diseased decaying roots 3 of the seedlings were diseased and 2 were healthy. In the pots containing healthy decaying roots 3 seedlings in one and 2 in the other became diseased in the fourth pair of leaves. Seedlings in fresh soil remained healthy.

In the laboratory no more cases of the disease developed in any of the seedlings for several weeks. The plants, however, made a slow growth, owing to the lack of sufficient light. After some time cases began to develop in all the pots containing decaying roots, and finally the 2 plants which had been transplanted to the fresh soil also became diseased. All of the plants in all the pots in the laboratory finally became diseased except the 4 seedlings in healthy soil. None of the plants in the laboratory are flowering, and all are unable to support their weight, falling down over the sides of the pots.

This evidence, along with that gathered from natural-sown seedlings, leads to the conclusion that the presence of decaying tobacco roots in the soil favors the development of the disease. The diseased roots are the most active in producing it, but the healthy roots also seem to favor its development. Whether this is due to the peroxidase set free in decay or to some other active substance can not be definitely decided. The direct infection experiments with this enzym, however, lead me to the hypothesis that it may be absorbed directly by young roots and thus serve, as in the case of hypodermic injections, to start that series of changes which results in the disease. It thus appears that the enzym obtained from either healthy or diseased plants is able, under the proper conditions, to produce the disease. There is no evidence that the peroxidase of the healthy and diseased plants is different, except that the latter is more active, both in the plant and out of it.

The evidence which I have collected, taken along with that obtained by other workers, especially Mayer and Beijerinck, is therefore very strongly in favor of the infectious nature of the trouble under certain conditions. The matter can not, however, be considered as settled. So far as the evidence at hand goes it appears that in growing cells there is possibly a definite relation between active oxidizing power, through the medium of oxidizing enzyms and the availability of reserve food to the growing cells. It appears that this balance between the oxidizing enzyms and the availability of reserve foods can be broken by removing on the one hand the supply of reserve foods, in any way during growth, in which case the enzym content of the cell is increased from two to four times the normal activity. This removal of reserve food may be either the result of diversion to other parts of the plant or by direct removal, as in the case of sucking insects, and possibly also can be brought about by other conditions not at present understood. The same pathological symptoms may therefore be brought about by very diverse causes. On the other hand, the most remarkable thing is that the introduction of the enzyms in question sets up the same series of pathological changes as is brought about by the removal of reserve food, namely, the increase of the normal enzyms of the cell, and the decrease of availability of reserve foods. When this pathological condition is reached it is very difficult for the plant to correct the trouble. The oxidizing enzyms probably move from one part of the plant to another, though how much of the general spread of the disease in the plant is due to such movement has not been determined.

The evidence of the communicability of this disease is quite as strong if not stronger than that upon which rests the belief in the communicability of ordinary variegation through grafting variegated on healthy plants. The two groups of diseases are at least very closely

related and are probably simply different phases of the same malady. Possibly peach yellows, as suggested by Smith, and the California vine disease belong here also and are to be similarly explained. Die-back of the orange may also be a related malady. A new disease of the vine, recently described as Le Court-Noue[a] by Professor Ravaz, shows all of the characteristic symptoms of a mosaic disease in marking of foliage, general stunting and deformity of leaves and branches, and transmission by grafting.

In a recent bulletin[b] I called special attention to the fact that plants rich in oxidizing enzyms were more sensitive to unfavorable conditions of temperature, moisture, and especially to insect enemies than plants poor in these enzyms. Some of the reasons for this greater sensitiveness I have already pointed out in this and other papers.[c] All through the work this observation has been confirmed not only for insect and animal pests, but for several kinds of fungi, especially the spot disease of tobacco and spot of violet. Czapek[d] has demonstrated a relation of oxidizing enzyms to geotropic curvature in root tips of several genera of plants. In the most sensitive condition of the young roots there is present, especially in the root tips, an oxidizing ferment and a reducing substance. As the root is stimulated to curve the reducing substance is increased in activity and the oxidizing enzym is reduced in its action.

This work of Czapek's suggests the possibility that there may be a relation of this kind between oxidizing and reducing substances in plants sensitive to parasites of various kinds, especially as I have already shown that sensitive individuals are rich in the oxidizing ferments. This presence of reducing substances is indicated in many of the preliminary tests, but their exact nature has not yet been determined. It appears quite likely that resistance to parasitic attack may be correlated with decreased irritability; or, what amounts in many cases to immunity may be brought about in the plant or animal by the development of these reducing substances. A further study of their relations in this connection is in progress in the laboratories under the writer's direction.

PREVENTIVE MEASURES.

The writer has not had opportunity, on account of other work, to go into the question of preventive measures under field conditions, to any great extent. The first work was to determine the nature and causes

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[a] L. Ravaz. Progres Agricole et Viticole, Nos. 21 and 24, 1900.

[b] Bul. 19, U. S. Dept. of Agr., Div. Veg. Phys. and Path.

[c] Physiology in Relation to Agriculture. Bull. 99, U. S. Dept. Agr., Office of Expt. Stations.

[d] Berichte d. d. botan. Gesellschaft, Bd. XV, pp. 516-520, 1897.

of the disease, the second step is to determine the remedy. In this inquiry the following facts must be remembered:

The disease is not due to parasites of any kind, but is the result of defective nutrition of the young dividing and rapidly growing cells, due to a lack of elaborated nitrogenous reserve food accompanied by an abnormal increase in activity of oxidizing enzyms in the diseased cells. The unusual activity of the enzym prevents the proper elaboration of reserve food, so that a plant once diseased seldom recovers. On the decay of the roots, leaves, and stems of both healthy and diseased plants, the enzym in question is liberated and remains active in the soil. The enzym is very soluble in water and appears to pass readily through plant membranes. If young plants take it up in sufficient quantity to reach the terminal bud, they become diseased in the characteristic way. Under field conditions there is little danger from infection in this manner, but in the seed bed the danger is much greater on account of the greater susceptibility of the young plants to disease, and the greater amount of free-oxidizing enzyms likely to be in the soil, due to the decay of roots and plants. New or steam-sterilized soil should therefore be used for the seed bed.

I have shown that transplanting, especially when the roots are injured, may produce the disease. Great care must therefore be taken not to injure the roots in this process or in subsequent cultivation, or to check the growth of the plants.

There is evidence that rapid growth, caused by too much nitrogenous manure or too high temperature, is favorable to the disease. Why this should be the case has not been determined. It is probably connected, however, with the manufacture of reserve nitrogen by the cells and its distribution to the rapidly growing parts.

Plants grown under such conditions are less able to stand successfully marked variations in temperature and moister conditions of soil and atmosphere. Variations of this kind favor the development of the disease in the less resistant plants.

Close clayey soils, packing hard after rains and requiring constant tillage, are not favorable to even growth of either the tops or the roots of tobacco plants. In moist, cloudy weather the plants will grow too fast, and in hot, dry weather the soil is likely to bake, checking growth and making probable injury to the roots in cultivation. Such soils are very favorable to the development of mosaic disease, as pointed out by Thaxter.[a] He found that loosening the soil by liming and giving partial shade, thus causing a more even condition of growth, very greatly reduced the amount of disease. The following table is taken from his report. It shows the percentage of "calicoed" plants on each plot, shaded and unshaded. "Calico" was very prevalent in all of the tobacco fields in the neighborhood.

[a] Conn. Report, 1899, III, p. 253.

Effects of liming and shading on development of mosaic disease.

Plat.	Amount of lime used.	Diseased plants on unshaded portion.	Diseased plants on shaded portion.
	Pounds.	*Per cent.*	*Per cent.*
I	300	10	5
II	500	17	2
III	1,000	0	2
IV	2,000	0	0

The plants under the partial slat shade did not grow quite as fast as those outside, but the conditions were, of course, less variable.

Crops grown under cheese-cloth covers protected at the side, thus making even conditions of growth, are said to be remarkably free from disease. The plants make a steady, rapid growth—much greater than in ordinary field culture.[a] The quality of the leaf is pronounced of the highest grade for cigar wrappers.

In the South, wet, poorly drained soil is said to favor the development of calico. This would be expected on the same grounds as suggested above.

Seed should not, on general principles, be saved from plants which develop this disease, though it is not as a rule transmitted in this way. The seed may lack in vigor, and the plants produced from them would be expected to have a tendency to develop the trouble in circumstances favorable to the malady. The experimental evidence on this point is, however, not conclusive.

The disease is not, so far as observed, produced by a lack of soil nutrients, though from its nature we would expect that a deficiency of nitrogen, phosphoric acid, lime, or magnesium might favor its development. Koning[b] says that manuring with kainit and Thomas slag diminishes the extent of the disease. Mayer, Beijerinck, and other investigators, however, agree that the trouble is not caused by the lack of any soil nutrients. It appears, so far as my own investigations go, that the trouble can not be cured by giving the plants additional food of any kind. Overfeeding with nitrogen favors the development of the disease, and there is some evidence that excess of nitrates in the cells may cause the excessive development of the ferments causing the disease. Very slight attacks of the disease known as mottled top are said not to injure the quality of the leaf to a sufficient extent to be noticeable commercially, though they are less elastic and have a poorer burn and aroma than healthy leaves.

It is hoped that the observations brought together in this paper may at least help those who are investigating this trouble to solve the problem of its control.

[a] See Report 65, U. S. Dept. of Agriculture.

[b] Zeitschrift für Pflanzenkrankheiten, 1899, p. 65.

D. G. PASSMORE.

A. HOEN & CO. LITH. BALTIMORE.

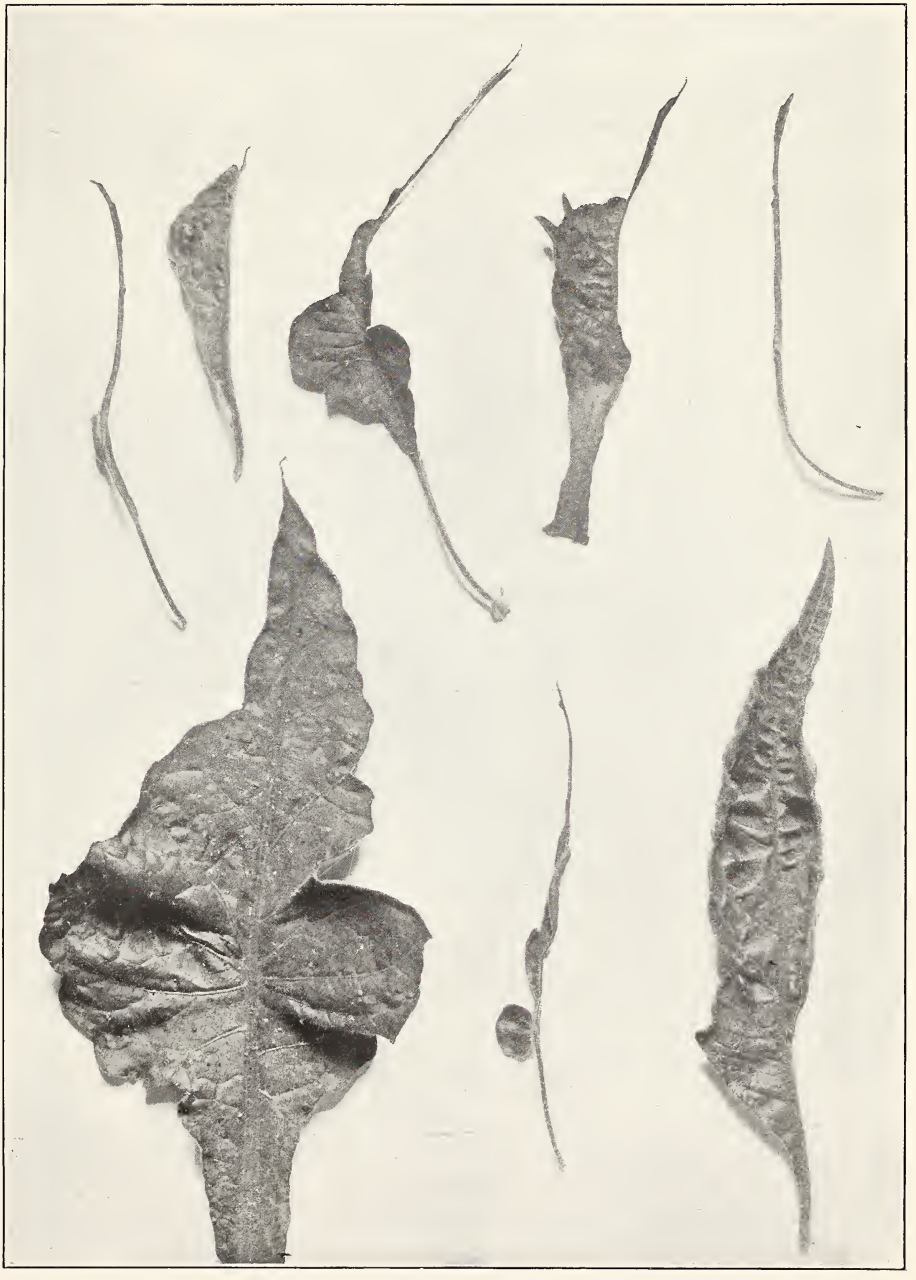
MOSAIC DISEASE OF TOBACCO:

(*a*) NATURAL DISEASE ON SUCKER, (*b*) DISEASE PRODUCED ON YOUNG PLANT BY CUTTING BACK.

D. G. PASSMORE.

MOSAIC DISEASE PRODUCED BY CUTTING BACK:
(*a*) POKEWEED, (*b*, *c*) TOMATO.

DISTORTION OF TOBACCO FOLIAGE BY MOSAIC DISEASE, CAUSED BY CUTTING BACK.



DISTORTED LEAVES FROM PLANT SHOWN IN PLATE III.

MOSAIC DISEASE OF TOMATO, PRODUCED BY CUTTING BACK. HEALTHY LEAF ON RIGHT.

D. G. PASSMORE. A. HOEN & CO. LITH. BALTIMORE.

MOSAIC DISEASE OF TOMATO:
(*a*) HEALTHY LEAF FOR COMPARISON, (*b* AND *c*) DISEASE PRODUCED BY CUTTING BACK.

BULLETINS OF THE BUREAU OF PLANT INDUSTRY.

The Bureau of Plant Industry, which was organized July 1, 1901, includes Vegetable Pathological and Physiological Investigations, Botanical Investigations and Experiments, Grass and Forage Plant Investigations, Pomological Investigations, and Gardens and Grounds, all of which were formerly separate Divisions, and also Seed and Plant Introduction, the Arlington Experimental Farm, Tea Investigations and Experiments, and the Congressional Seed Distribution. Beginning with the date of organization of the Bureau, the independent series of bulletins of the Division of Vegetable Pathology and Physiology, the last number of which was 29, and of each of the other Divisions were discontinued, and all are now published as one series of the Bureau.

The bulletins published in the series are:

No. 1. The Relation of Lime and Magnesia to Plant Growth. 1901.
2. Spermatogenesis and Fecundation of Zamia. 1901.
3. Macaroni Wheats. 1901.
4. Range Improvement in Arizona. 1901.
5. Seeds and Plants Imported through the Section of Seed and Plant Introduction, Inventory No. 9, Nos. 4351–5500. 1902.
6. A List of American Varieties of Peppers. 1902.
7. The Algerian Durum Wheats: A Classified List, with Descriptions. 1902.
8. A Collection of Economic and other Fungi Prepared for Distribution. 1902.
9. North American Species of Spartina. 1902.
10. Records of Seed Distribution and Cooperative Experiments with Grasses and Forage Plants. 1902.
11. Johnson Grass: Reports of Investigations made during the Season of 1901–1902.
12. Stock Ranges of Northwestern California. 1902.
13. Experiments in Range Improvement in Central Texas. 1902.
14. The Decay of Timber and the Methods of Preventing it. 1902.
15. Forage Conditions on the Northern Border of the Great Basin. 1902.
16. A Preliminary Study of the Germination of the Spores of Agaricus campestris and other Basidiomycetous Fungi. 1902.
17. Some Diseases of the Cowpea. 1902.